Commonwealth of Pennsylvania

BULLETIN No. 8.

Department of Fisheries

Continued Observations and Experiments on the so-called Thyroid Carcinoma of Brook Trout (Salvelinus Fontinalis) and its Relation to Ordinary Goitre

By DAVID MARINE, M. D. *and*

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Cleveland, Ohio

From the H. K. Cushing Laboratory of Experimental Medicine,
Western Reserve University, in Conjunction with
the Pennsylvania State Department
of Fisheries

W. E. MEEHAN, *Commissioner of Fisheries*

Issued May 1, 1911

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INTRODUCTION

A year ago the Department of Fisheries published a bulletin entitled "Thyroid Carcinoma of Brook Trout," the result of investigations made by Dr. David Marine and Dr. C. H. Lenhart of the Western Reserve University, Cleveland, Ohio, among Pennsylvania trout and by the authority of the Department.

The investigations made by these two highly qualified men indicated strongly that goitre in fish did not necessarily mean that cancer would follow. The investigations were not considered by any means as complete and Doctors Marine and Lenhart continued their observations and experiments throughout last year and the results are given in this bulletin. They are strongly confirmatory of the original investigations. Owing to the importance of the subject the paper was read with my consent before the Cleveland Academy of Medicine February 10, 1911. An exceedingly important outcome of this investigation is the fact that by it practical suggestions are possible to be made to prevent the spread of the disease and get rid of that which may exist.

W. E. MEEHAN,
Commissioner of Fisheries.

Further Observations and Experiments on the So-called Thyroid Carcinoma of the Brook Trout (Salvelinus Fontinalis) and its Relation to Endemic Goitre

By DAVID MARINE, M. D. *and*
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From the H. K. Cushing Laboratory of Experimental Medicine, Western Reserve University, in conjunction with the Pennsylvania State Fish Commission.

TABLE No. 1—Histological Condition of Thyroid.

No. of fish.	Duration of Iodin, R.	Histological Condition of Thyroid.			Epithelium.	Diagnosis, Remarks, Etc.
		Distribution Infiltration.	Stroma.	Stainable Colloid.		
1	40 days.	Large ulcerated goitre all tissues beneath pharyngeal mucosa infiltrated. Disappearance of bone and muscle.	Markedly thickened throughout and densely infiltrated leucocytes areas of abcess formation.	In the more protected areas just beneath pharyngeal mucosa and along the gill arch arteries are areas with well stained colloid and but few leucocytes.	Flat cubical in the involuted areas. Little distinguishable thyroid tissues on account of the inflammatory exudate. One papillo-adenoma not affected by iodin.	This is an example of a severely ulcerated goitre with marked general inflammatory reaction, giving appearance in places of sarcoma. Most of thyroid follicles filled with leucocytes. Involution, general infection and enclosed papillo-adenoma.
2	40 days.	Large ulcerated goitre considerable thyroid tissue throughout which has extended into all subpharngeal tissues absorption of bone and cartilage compression of aorta.	Everywhere thickened and densely infiltrated with leucocytes.	The well preserved follicles contain colloid. Entire lower portion an infiltrated mass with much scar tissue formation.	Cubical in the preserved portions while in the infected area epithelium is not clearly distinguishable.	Old markedly inflamed goitre with destruction of most of the true thyroid tissues by the inflammatory processes.
3	40 days.	Large, fattened nearly healed goitre, all subpharyngeal tissues infiltrated absorption of bone muscle and cartilage. Compression of aorta. Thyroid growth at tip of lower jaw.	Increased throughout especially in the exposed parts.	Throughout goitre. The colloid is normal. Leucocytes in the colloid of the inflammatory area.	Cubodal in general but columnar on the crests of some of the infoldings and particularly in the papillomatous area.	Rapidly involuting goitre with healing of the infection. There is an adenomatous area that is beginning to involute. The submaxillary thyroid tissue has involuted to pure colloid state.

4	40 days,	Large ulcerated goitre. All subpharyngeal tissues infiltrated.	Everywhere increased. Much cellular exudate throughout ventral portion of goitre.	All the protected areas contain colloid. Those in the inflammatory area are well defined and filled with leucocytes.	In general cuboidal in the incomplicated portions but columnar in the inflamed area.	Example of involution to the colloid state occurring in a mildly infected goitre. Infection resolving.
5	40 days,	Small non-ulcerated goitre or rather healed goitre. All tissues invaded. Absorption of bone.	Markedly increased throughout, probably inflammatory scar tissue.	Follicles small colloid containing still few leucocytes in some follicles.	Cuboidal throughout,--	Example of partially complete involution with healing of the ulcerated and infected area.
6	62 days,	All subpharyngeal tissues involved. Little normal thyroid present. Absorption of bone and compression of aorta.	Scar tissues overgrowth. Inflammatory exudate throughout stroma.	There are colloid containing follicles scattered throughout dorsal part of goitre.	Cuboidal in the colloid containing follicles. Much of the thyroid tissue replaced by cellular exudate and scar tissue.	Example of slight involution present when the inflammatory reaction is very marked, even in the 62 days. Illustrates the disturbance in the normal reaction caused by infection.
7	62 days,	Small healed goitre, all subpharyngeal tissues invaded, absorption of muscle and bone.	Everywhere increased, especially in the ventral portions.	Follicles generally small, colloid containing throughout.	Cuboidal regular and uniform.	Example of complete healing of infected area and of complete involution.
8	62 days,	Healed goitre. All subpharyngeal tissues invaded.	Very dense, probably result of old infection.	Follicles very small contain normal colloid. One area with marked infoldings and plications and containing very little colloid like material.	Cuboidal throughout the involuted portion. The adenomatous area is still high columnar.	Example of involution and resolution of an infected goitre containing an adenoma with Papillomatous ingrowths but slightly modified by iodin in 62 days.
9	62 days,	Healed goitre. All tissues beneath mucosa invaded and also small nodules projecting into pharynx. Absorption of bone and muscle.	Slightly increased	Follicles enlarged. Contain colloid some exudate in stroma in places.	Cuboidal.	Example of healing and involution of pure type of goitre where only complication was infection and ulceration.

TABLE No. 2—Histological Condition of Thyroid.

No. of fish.	Duration of iodin treatment.	Histological Condition of Thyroid Coland.			Epithelium.	Diagnosis and General
		Distribution, Infiltration.	Stroma.	Stainable Colloid.		
1	40 days.	Entire space beneath pharyngeal mucosa infiltrated with thyroid tissue. Absorption of bone, cartilage and muscle, ventral surface of goitre worn away by friction and ulceration.	Generalized increase, also extensive celular exudate and granulation tissue formation in stroma.	Many follicles with normal colloid throughout goitre. Extensive areas where follicles contain shed epithelium and leucocytes with little true colloid.	Flat cuboidal in all follicles with normal colloid columnar in the inflammatory areas, one area with arborescent outgrowths of lining epithelium (tumor).	Involution to colloid state well advanced. The ulceration and inflammatory reaction are complicating and retarding factors one large circumscribed papillomatous area (adenoma?)
2	40 days.	Entire space beneath pharyngeal mucosa infiltrated with thyroid tissue including bone and cartilage.	Slightly increased especially about bone and skin.	Follicles generally enlarged and filled normally staining colloid.	Uniform, flat cuboidal.	Complete involution to colloid state. No complicatory factor.
3	40 days.	Entire space beneath pharyngeal mucosa infiltrated with thyroid tissue, ulcerated.	Marked increase in stroma and extension of inflammatory reaction throughout goitre.	Follicles in less infected portion are filled with colloid. In the main mass of the goitre the follicles contain leucocytes and granular albuminous debris.	Throughout more affected areas, the follicles have columnar epithelium, while remaining ones have cuboidal epithelium.	Involution well advanced in the areas free from inflammatory reaction. No papillomatous areas.
4	40 days.	Entire subpharyngeal space invaded with thyroid tissue including bone and muscle.	Slight general increase.	Follicles enlarged. Show infoldings of the lining epithelium. Colloid normal.	Cuboidal throughout...	Complete involution of a small uncomplicated goitre.
5	62 days.	Entire space beneath pharyngeal mucosa invaded and infiltrated with thyroid tissue.	Well marked increase,--	Follicles small rounded and filled with colloid.	Cuboidal, ...	Complete involution. No complication.

6	62 days,	Small nodular growths of thyroid tissue elevating the pharyngeal mucosa. All tissues beneath mucosa invaded. Extensions along gill arch arteries.	Slightly increased, ------	Follicles enlarged filled with colloid.	Cuboidal, ----------	Complete involution to colloid state. No complication accessory thyroid growth at tip of lower lip showing compiete involution.
7	62 days,	Entire subpharyngeal space filled with thyroid tissue erosion of bone and cartilage.	Increased around the more resistent structures.	Follicles large in the body of the goltre but small and separated by stroma bands at periphery. Colloid normal.	Cuboidal, ----------	Complete involution, no complication, some evidence of a previous mild infection.

INTRODUCTION.

In our first report, Bulletin No. 7, Department of Fisheries, 1910 (Jour. Exp. Med. 1910, XII, 311) on this subject the following observations were emphasized:—

(1) That all the trout, irrespective of age or location in the ponds of the hatchery, were affected with thyroid hyperplasia.

(2) That it was most marked in the younger fish.

(3) That a definite relation existed between the amount of water, the concentration of fish and the quantity of food, and the severity of the disease.

(4) That older trout are less susceptible to the disease as shown by a tendency towards spontaneous recovery under constant conditions as regards the concentration of fish, the water supply and the food.

(5) That recovery from the less marked degrees of thyroid hyperplasia constantly occurred either on taking them from the ponds and putting them in their native environment (the brook) or on leaving them in the theoretically polluted pond water, but reducing the food and the concentration of fish or on the addition of small amounts of iodin.

(6) That our attempts to transplant the hyperplastic thyroid tissue from one fish to another of the same age and stock were negative.

(7) That anatomical studies alone were insufficient criteria upon which to make a diagnosis of cancer because the thyroid of the bony fish being normally a widely and loosely distributed, non-encapsulated gland—any growth of which would give the appearance of invasion of the surrounding tissues.

(8) That there was no experimental basis for considering the disease, in its early stages at least, other than severe, endemic goitre.

In the following report we shall present our observations on the earliest manifestations of thyroid hyperplasia in the very young fish; our observations on the spontaneous recovery of the affected fish when transferred to a natural environment; and our experiments with the late stages of the disease, or those stages associated with large, visible and ulcerated goitres.

These observations and experiments were made during the summer of 1910 at the same hatchery where the earlier work was done.

OBSERVATIONS ON THE HISTOLOGICAL CONDITION OF THE THYROIDS OF TROUT FRY.

In order to determine how early in the life of hatchery trout thyroid hyperplasia (goitre) began, we collected specimens of the fry weekly, beginning as soon as the egg membranes were shed (Jan. 15, 1910) and continuing until Oct. 8, 1910.*

Sections have been prepared from the thyroid areas of the entire series. It is difficult to distinguish the thyroid tissue in these fry up to the end of the second month, and we would hesitate to state that the thyroids at this age were hyperplastic, although there is no colloid present and the epithelium lining the follicles is high cubical. In fortunate sections it is possible to distinguish an alveolar lumen. Beginning with sections taken March 16, one can positively state that hyperplasia has begun. The thyroid tissue in most cases fills the areolar space about the ventral aorta. The epithelium lining the follicles is high cubical, regular in type with bluish tinted cytoplasm. There is no stainable colloid. The first evidence of infiltration or invasion of the surrounding structures is seen in the specimens taken on April 30. The thyroid follicles have filled the aortic space and have extended around the lateral muscle bundles and dorsally toward the floor of the pharynx. From this age (3½ months) the growth is progressive, extending into the more resistant structures, as striped muscle and bone. As the gland follicles grow into the pharyngeal submucosa the vascularity of the newly formed thyroid tissue is transmitted through the translucent mucosa of the pharynx as a pale red discoloration of the floor of the pharynx between the first and third gill arches. This pharyngeal discoloration was detectable in these particular fry about the middle of July (6 months old). It may occur earlier in fish kept under less favorable conditions. The appearance of visible goitres is always a late manifestation, and we have never observed it until the subpharyngeal tissues were filled with thyroid tissue. Gaylord (Jour. Am. Med. Assoc. 1910 LIV 227) has observed visible goitres in brook trout fry of about 6 months old. We

*The entire series was collected from a single cypress hatch trough. This was one of a series of 8—all of the same size (13 feet long, by 20 inches wide, by 6 inches deep). Water was supplied direct from the spring in sufficient quantity to replace the contents of the trough every 20 minutes. These troughs were cleaned every day. The fry were fed 4 times daily for the first month with an aqueous suspension of hog's liver strained through muslin. During the second and third months a similar suspension strained through coarser muslin was fed. Beginning with the fourth month they were fed finely ground hog's heart muscle freed from adventitious tissues. As the fish grew, the heart muscle was ground coarser. No estimation was made of the number of fry in the trough at a given time. They were thinned from time to time and though always crowded had free swimming space.

have seen goitres in brook trout fry of about 7 months old. This is not common, however, and indicates unusually favorable conditions for thyroid growth and hence unfavorable conditions of food and environment.

The results of this study show that thyroid hyperplasia begins at a very early age and is detectable at the end of the second month of extraoval life. These fry developed thyroid hyperplasia while living in unused water direct from the spring and under unusually clean surroundings as regards any gross appearance of filth.

The crowding and the food therefore seem to be the most important factors, and the food would appear to be more important than the crowding. The influence that crowding may have on the nutrition of the fish is at this time purely speculative. It would seem that its solution may lie in the field of biochemistry, either by the detection of some substance developed under such conditions of food, environment and overcrowding which the thyroid growth attempts to counteract, or by showing that the water contains salts necessary for the normal nutrition of the fish in sufficient amounts under natural conditions but insufficient under these highly artificial conditions.

The influence which food may have is also at this time speculative, as little is known of the food requirements of these fish. Liver or heart muscle is a highly abnormal diet and is most likely deficient in many of the elements necessary for normal nutrition. When fed in excessive amounts it is conceivable that it may be injurious from the excess of certain elements as well as from the deficiency of other elements. Iodin has a preventative and curative effect on these early hyperplasias. The explanation of the action of iodin in this role would probably explain the cause of thyroid hyperplasia. It may be stated that there is a deficiency of iodin in all thyroid hyperplasias, but it is not known whence it originates, or what the underlying factors which bring it about may be.

OBSERVATIONS ON THE HISTOLOGICAL STATE OF THE THYROID GLANDS IN A SERIES OF FISH REPRESENTING SPECIMENS FROM ALL TANKS (PONDS).

Similar observations were made in October, 1909, when the ages of the fish were 32, 20 and 8 months respectively. Now (June, 1910) the ages are 29, 17 and 5 months—the 3 years old fish having been transferred to the trout stream in April, 1910, and the 2 and the 1 year old fish moved down to take their places and make room for the

1910 fry. Apart from the different ages of the fish and their changed location there are but two general conditions markedly different from those obtaining in 1909:—(1) season (summer vs. autumn) (2) a decreased water supply owing to the pipe line not being in operation for the past 6 months. It was thought that this second examination might offer additional data concerning any seasonal influence or the effect of a lessened water supply, and also serve as a control for the observations made in October.

The conditions as revealed by the histological examination of 105 trout thyroids showed that they all had well marked hyperplasia. The fish thyroids of the upper division (Houses I-VI see Fig. 1) were less hyperplastic than at the first examination in October, while the thyroids of the lower division (Houses VII-XI) were more markedly hyperplastic than in October.*

The food both as to quantity and quality was the same as last year. The crowding of the fish was probably not so great. The water supply to the first 6 houses of the series was increased probably to a third more than that available in October. This was due to a decreased flow from the spring during the months of July, August, September and October. We believe the increased water supply during the winter and spring months largely explains the better condition of the fish thyroids in Houses I-VI in June than that which existed in October, 1909.

In the lower division (Houses VII-XI) it will be recalled that in October with the pipe line in operation the thyroids were undergoing spontaneous involution, while in June with the pipe line out of operation for the past 6 months the thyroids in general were more hyperplastic than those of the upper division, with the exception of House VII, which is the first house the water enters after its passage along the original brook bed from the upper division, and to which a small spring is added. In this house (VII) the thyroids are noticeably less active as judged by the standards above mentioned, and it is our opinion that the passage of the water along the brook and its slight increase in volume favorably alters in some unknown manner the effect on the fish. We also look on the absence of the additional water supply from the pipe line for the past 6 months as an important factor in bringing about the much more active thyroid hyperplasia present throughout the lower division than existed at the examination in October. There was no evidence of any noteworthy seasonal effect to be made out. It is generally stated that in mammals the thyroid tends to be less active (hyperplastic) during the winter months.

*The degree of hyperplasia is judged by the amount of stainable colloid and the size of the epithelial cells and not by the total amount of thyroid tissue or its extension into the surrounding structures, which is present in all of these hatchery fish.

Summing up these observations one may say that they offer further evidence that increasing or decreasing the water supply modifies the condition of the thyroid. We are still ignorant of how an increased water supply lessens the thyroid hyperplasia. It may be that the salts in solution are necessary for normal nutrition and that their reduction disturbs the physiological balance, or that the susceptibility to some infectious agent is decreased by dilution, or by improving the fishes' general nutrition.

OBSERVATIONS ON THE HISTOLOGICAL CONDITION OF THE THYROIDS OF THE TROUT OBTAINED FROM THE RUNWAYS ABOVE, BETWEEN AND BELOW THE SEVERAL TANKS (PONDS).

Of this series of 25 trout varying in age from 17 months to 4 years, 5 were taken from the brook above all houses, 16 from the runways within and between the 11 houses, and 4 from the tailrace below all houses. Of the 5 taken from the brook above all houses, one was 29 months old and 4 were 17 months old. They were normally colored, strong, vigorous fish and had spent their entire lives in this space of about 50 yards. All had normal thyroids both as regards the individual units—the follicles—and the total amount of thyroid tissue. They had not been fed or cared for in any way.

Of the 16 fish taken from the wooden runways within and between the several houses, 7 were considered as being 17 months old, 3 as 29 months old, and 6 as being over 3 years old. None of these fish had normal thyroids. In all there was more or less extensive spreading of the thyroid tissue beyond the normal area. In 11 of the fish the thyroids were in the colloid or resting stage i. e. the individual follicles contained normally staining colloid and the epithelium was normal in type, although the total number of follicles was greatly increased above the normal. The remaining 5 fish had mild degrees of active hyperplasia. These 16 fish had escaped from the ponds, but there was no way of ascertaining how long they had lived in the runways except by their size and the development of the color pigment in the skin, which indicated a stay of many months, and certainly of years in some instances.

The 4 trout taken from the tailrace below the last house were fish that had been transferred to the trout stream not later than April 15, 1910, and had therefore spent at least 2 months and perhaps longer, outside the ponds. All were large, 3 years old fish with darkly pigmented bodies. Two of these had pure colloid goitres and in the remaining two involution was not complete.

Summing up the observation in this series, it is seen that the trout living in the raceway *above* all houses and which had never been confined in the ponds or fed or cared for in any way, developed normally and maintained normal thyroids throughout their lives, while fish not a yard away, but confined in the ponds, artificially fed and overcrowded, quickly developed marked thyroid hyperplasia.

Next it is seen that fish taken from the small plank troughs which conduct the water from one pond to another may have colloid or quiescent thyroids, that is, may recover from the hyperplasia they had at the time of their escape from the ponds, even though fish in ponds above and below them are at the same time developing thyroid hyperplasia. Such observations do not accord with our present conceptions of a water-born infectious agent as the etiological factor. On the other hand certain of these fish had active hyperplasia, showing that the same factors which operate within the ponds are also operative in the raceways, but to a lesser degree.

Lastly fish taken from the tailrace show the same tendency toward recovery, although living in the theoretically most polluted water. No general conclusion can be drawn from these observations beyond the negative evidence against a water born infection as the cause of goitre, and in favor of the interaction of several factors of a chemical nature influencing the nutrition of the fish.

OBSERVATIONS ON THE HISTOLOGICAL CONDITION OF THE THYROIDS OF THE TROUT TAKEN FROM THE TROUT STREAM.

There were 18 trout in this series. All of these fish had spent at least 3 months in the stream. Two of them had the gross appearance of native trout, and as their thyroids were histologically normal, it may be concluded that they had never been confined in the ponds. The remaining 16 fish had enlarged thyroids. Three had small external goitres. In most of the cases the thyroid had returned to the pure colloid state, but in certain specimens the involution was not complete.

To sum up this series, one sees that fish hatched and reared in the stream do not develop thyroid hyperplasia, and also that fish whose thyroids underwent marked hyperplasia during their stay in the ponds (without exception so far as our observations on 52 cases are concerned) undergo spontaneous recovery when transferred to the trout stream. We have also examined pickerel, suckers and cat fish from this stream and have never observed abnormal thyroids in them.

PART II.

EXPERIMENTAL OBSERVATIONS.

These experiments were undertaken to supplement the experiments made in October, 1909, when the effect of iodin on mild degrees of hyperplasia* were studied, and to extend these observations to include the severest grades of goitre (i. e. fish with large, ulcerated, ventral goitres).

They comprise three groups of observations, viz:

(1) The effect of iodin on 41 months' old fish, with large, ulcerated ventral goitres and with general emaciation.

(2) The effect of iodin on 29 months' old fish with small, visible goitres, but otherwise of a clean, healthy appearance.

(3) The effect on 29 months' old trout with small, ventral goitres of living in the trout stream without the administration of iodin, as judged by their subsequent thyroid conditions.

EXPERIMENT NO. 1.

EFFECT OF IODIN ON THE LARGE, ULCERATED GOITRES.*

The experiment was carried out as follows:—Twelve 41 months' old, emaciated brook trout, which had been saved for this purpose (instead of being destroyed, as is usually done) when the three old fish were transferred to the trout stream in April, were placed in

*The first constant and readily detectable clinical sign of thyroid hyperplasia is the reddish discoloration of the floor of the pharynx. This reddening depends on the extension of the highly vascular thyroid tissue up to the mucosa of the pharynx and the transmission of the color of this highly vascular tissue through the translucent mucosa. The extent of this area of reddish discoloration (the color of the normal pharyngeal floor being white or grayish-white) is a good clinical index of the extent of the thyroid overgrowth. As the hyperplasia involutes to the colloid or quiescent state during recovery, the vascularity decreases and with it the reddish color fades to a yellowish or yellowish-brown appearance and persists throughout the life of the fish for the reason that the thyroid follicles with their colloid contents, upon which the color depends, persists.

*Ulceration is a very common complication in these large goitres. The most important causes predisposing to infection are:

(1) Injury. The goitre being the most prominent point on the ventral surface of the fish, it is exposed to frequent trauma, especially in the confined quarters of the ponds.

(2 As the goitre enlarges the skin over it is thinned by pressure atrophy, and perhaps the break in the continuity may occur from this cause alone. When once the skin is broken, infection is practically unavoidable, first on account of the rich bacterial flora of the ponds and secondly on account of the increased susceptibility of these fish to infection which the growing goitre implies. After infection occurs the necrotic tissue is worn away by friction, so that as we see these goitres they often have flat ventral surfaces practically in a plane with the ventral surfaces of the bodies of the fish.

Therefore it can be seen that by placing such fish in shallow troughs for experimental purposes the chances of healing are reduced, because the exposed surface of the goitre is frequently rubbed against the bottom. The occurrence of infection in fish goitre is perhaps the most important and most frequent complication, and should be considered in any interpretation of histological data.

a cypress trough 13 feet long, 20 inches wide, and 8 inches deep, on June 20. The water used was taken directly from a spring marked X on the diagram (see Fig. 1). Its temperature was 48-50 degrees Fahrenheit. The flow through the trough was adjusted so that it was sufficient to fill the trough in 1 hour. All the fish had large ventral, ulcerated and bleeding goitres. Three had gill fungus and died during the experiment. Of the remaining nine, five were sacrificed on the 40th day, and four on the 62d day. The food consisted of about 3 oz. of finely chopped hog's heart or liver each morning and on three occasions during the first 40 days live minnows were given. One cc. of the U. S. P. Tincture of Iodin (KI 5 grams, I 7 grams, Alcohol (95 per cent.) to 100 cc. was added to the trough contents each morning before feeding. The trough was located in a well shaded gully and wire cloth was fastened about it to prevent the fish from jumping out. Once each week the debris, leaves, uneaten food and feces was removed from the trough by flushing. The examination of the nine fish at the time of removal revealed remarkable changes in their appearance. They had all gained greatly in weight. They had lost much of their dark pigment, and the color pigment had developed in the fins and along the sides of the fish. They were lighter in color than the native trout, having the silvery sheen approaching that of the true salmon. They were all robust, clean fish. Of the 5 removed on the 40th. day three still had visible, flattened and ulcerated goitres. Of the 4 removed on the 62nd day only one had distinct ulceration—the others having practically healed.

The histological condition of the thyroids of these nine trout is given in table No. 1. Of the five removed on the 40th. day all showed evidence of infection. In two it had nearly resolved. The stroma was increased in all, probably abnormally so on account of the infection. In the more protected follicles to which the infection had not extended, or in which it had cleared up, there was the normal accumulation of colloid with the return of the follicular epithelium to the flat cubical type. Throughout the infected areas the follicles contained large numbers of leucocytes and shed epithelial cells, and the stroma was cellular, giving the appearance of much new connective tissue formation (granuloma) together with an infiltration of leucocytes. This appearance at first gives one the impression of a connective tissue tumor rather than of thyroid granulation tissue. In two there were somewhat circumscribed areas with very striking papillomatous outgrowths and foldings of the lining epithelium. In one of these tumor nodules iodin had had no detectable effect toward involution, while in the second and less extensive papillomatous growth iodin had stopped its growth and brought about the early changes of involution, i. e. beginning colloid formation and shrink-

age in the size of the epithelial cells. These more or less circumscribed areas of rapidly growing thyroid tissue, with a tendency toward arborescence, are frequently seen in fish goitres, and, as they have many of the attributes of true thyroid tumors (localized, rapid growth, atypical follicle developments, and slight or no reaction with iodin), we look upon them as true tumors; and, until something suggestive of malignancy can be recognized, as benign tumors comparable to the ordinary tumors in human goitres. Of the four trout removed on the 62nd day, one had a papillomatous tumor with the surrounding original hyperplasia completely involuted, while the tumor was only slightly modified, again suggesting that in such cases the thyroid cells have lost certain of the attributes of ordinary hyperplastic thyroid cells. All of these 62 days' fish thyroids showed practically complete involution, with nearly complete healing of the infections. Summing up this experiment, the more striking features are: (1) the remarkable improvement that may occur in these fish in a short time; (2) the extent and frequency of infectious processes in fish goitres, and the influence which they exert in retarding involution with iodin; (3) the presence of adeno-papillomatous areas in 3 of the 9 cases that resemble in many respects true thyroid tumors. These 9 cases represent the very advanced stage of fish goitre. Such fish are always destroyed by the care taker when the "transfers" are made, because it is popularly believed that they cannot recover. This is wrong. They can and do recover in most instances where sufficiently favorable hygienic conditions are instituted. The goitre does not ordinarily disappear as the fish recovers for the same reason that it does not disappear with recovery in mammals. It does, however, involute to its colloid or quiescent stage just as in mammals, and this is the nearest normal state to which such thyroids may return. There is, therefore, no biological evidence that these severe grades of goitre are cancerous.

EXPERIMENT NO. 2.

EFFECT OF IODIN ON THE THYROID HYPERPLASIA OF 29 MONTHS' OLD FISH WITH SMALL, VENTRAL GOITRES BUT OTHERWISE OF HEALTHY APPEARANCE.

This experiment was carried out exactly as No. 1. The trough was of the same size and situated along-side of that used in Experiment No. 1. The water supply both as regards quality and quantity, the food, the amount of iodin and the general care were similar. Seven

trout were placed in the trough in June 20, 1910. Four were removed on July 30 and three on Sept. 1, 1910. They all gained greatly in weight. Much of the dark skin pigment had disappeared. The orange colored pigment in the fins and the yellow-red spots along the sides of the body had developed. The pharyngeal mucosa had lost much of the original reddish discoloration, and the ventral goitres, which at the beginning of the experiment varied in size from 3-6 mm. in diameter, became much smaller and of a yellowish translucent appearance—showing that the fish goitre in common with the mammalian goitre usually becomes smaller as the involution to the colloid state takes place. Two trout had ulcerated and infected goitres, probably as the result of rubbing against the trough walls.

The histological condition is tabulated in Table No. 2, and may be summarized as follows:—In all the specimens the thyroid tissue had infiltrated all the structures beneath the pharyngeal mucosa. There were extensions into the gill arches along the arteries. The adjacent muscle in some cases was totally replaced by thyroid tissue. There was much absorption of the adjacent bony and cartilagenous tissues. While the thyroid tissue completely surrounded the aorta, there was no case where it had extended beyond the adventitial coat. There was often compression of the aorta and its branches by the adjacent thyroid tissue. In the two infected specimens there was a very marked increase in the stroma, which was probably largely of inflammatory origin. The stroma is normally increased in active hyperplasia, being more pronounced about the more resistant structures as the bone, skin and arteries. Histologically, with the exception of the ulcerated goitres, the folloicles all contained normally staining colloid. The epithelium had returned to the normal cubical form, and the blood supply was reduced. In one case there was a small accessory goitre at the tip of the lower lip, which upon histological examination showed complete involution to the colloid state, just as was the case of the thyroid proper. This is the best or biological evidence that these submaxillary growths are not metastases, but embryologically misplaced thyroid *anlagen*.*

Taking the experiment as a whole, we find that these mild degrees of visible goitres are affected by iodin in the same way as the still earlier stages, before visible goitres appear, and hence are not true tumors but merely the more advanced stage of the common process-goitre.

*We have never observed true thyroid metastases. The submaxillary growths have been looked upon as metastases by some observers. They were present in 2-3 per cent. of all fish with visible ventral goitres examined by us. We have observed one instance of an abdominal goitre in a 29 mos. old fish. The thyroid mass was round, encapsulated and measured 1 x 1.2 cm. It was attached to the cardiac end of the stomach by a connective tissue pedicle. This fish also had a large ventral goitre histologically identical with the abdominal growth. Careful examination of the visceral organs did not show any thyroid tissue. We therefore looked upon this specimen as an enlarged aberrant thyroid just as the submaxillary growths.

The question of control experiments in the case of trout cannot be carried out as in mammals for the reason that we cannot control the hemmorrhage and infection consequent upon removing portions of the goitre. We therefore took as controls 5 fish of the same age and from the same pond with small ventral goitres. The histological examination of these specimens showed well marked active hyperplasia in all.

EXPERIMENT NO. 3.

EFFECT ON THE THYROID GLANDS OF 29 MONTHS' OLD FISH (TROUT) OF A RESIDENCE OF 38 DAYS IN THE TROUT STREAM.

The experiment was carried out as follows:—A pond was made in the trout stream (location marked * in Fig. 1) by fencing off with wire cloth a portion of a pool 40 feet long where an overhanging bank afforded cover and shade for the fish. This also allowed free entrance and exit for the passing water, and closely approximated the natural environment of the native trout. Fifteen 29 months' old fish with very small ventral goitres, but otherwise of a healthful appearance, were transferred on June 22 from the ponds to the enclosure above described. No iodin was used and the only food directly given were some live minnows on three occasions. On July 30 we were able to recover 8 of these trout. Their general color was perhaps somewhat brighter than at the beginning of the experiment, and there had occurred a slight developemnt of the orange and yellow skin spots. All were active, clean, vigorous, well nourished fish. The pharyngeal mucosa was still somewhat reddish in color and the ventral tumors were still visible.

Histological examination of these 8 fish showed the usual generalized invasion of all the subpharyngeal tissues, with extensions to the gill arches. The follicles were in general large, but about the more resistant structures where the stroma is normally dense they were small. All the follicles contained stainable colloid, but in none had it reached its normal staining properties. The epithelium lining the follicles was still columnar or high cubical. When these fish were compared with the controls, however, it was found that a great change had taken place in the 38 days. That is, the thyroids were involuting to the colloid stage, but the process was still far from complete. This slow change was to be expected since we had uniformly found the same changes occurring in the thyroids of the trout taken at random from the trout stream after their residence in the stream for varying lengths of time.

When this experiment is considered in connection with experiments one and two, it is seen that spontaneous involution (recovery) of the thyroid is much slower than induced involution with iodin. It will be recalled that the same time differences between spontaneous involution and induced involution with iodin are present in mammals. but that the end stage of each process, viz.—the colloid gland—is anatomically and physiologically identical.

SUMMARY.

In a series of observations upon brook trout thyroids extending from the time of hatching to fish of 4 or more years old we have been able to follow step by step the development of thyroid hyperplasia. In these hatchery trout, thyroid overgrowth may be recognized anatomically at the end of the second month of extra-oval life. Under the conditions in which these fish live the thyroid overgrowth progressively increases, at first filling the aortic space and then invading the surrounding bone and muscle. In our series this extension into the adjacent tissues was not recognizable until the 4th month of extra-oval life. The time when invasion of the surrounding tissues is first noticed is of course subject to great variation in different years and different hatcheries, depending on the general hygienic conditions, food, water supply, etc. If the growth continues (which it usually does) throughout the rapidly growing period of the trout it may be first detected, clinically, by a reddening of the pharyngeal floor as the thyroid tissue spreads to the submucosa. Later definite external goitres appear ventrally. These may be present as early as the 6th month of life. The thyroid being most active during the growing period of the fish (just as in mammals), the greatest number of visible goitres appear during the 2d. and 3d. years of life. In older fish the thyroid again becomes less active and there is a tendency toward spontaneous recovery. There is no noteworthy change in the anatomical appearance of the thyroid growth, whether seen in the very young or in older fish, other than that clearly dependent on the age of the fish, mechanical factors, as size of the goitre, and complicating factors, as infection, hemorrhage, degeneration, etc. There exists therefore no anatomical basis for the diagnosis of cancer in the older fish that is not also present in the youngest fish with thyroid hyperplasia. All the appearance of invasion, atypical cell growth, etc., are the results of the progressive growth and consequent extension of a nonencapsulated epithelial tissue along the paths of least resistance.

In many of the larger goitres there are more or less distinct areas of thyroid hyperplasia histologically different from the surrounding thyroid tissue, which we look upon as benign tumors comparable to the benign tumors seen in human goitres. We class them as tumors because they do not react with iodin as does ordinary hyperplasia. To call these tumors cancer is going beyond our present knowledge—even though from analogy with mammalian tumors one might expect certain ones to continue on to true carcinoma. The most common type is the arborescent—papillomatous form and its many modifications.

Infection is a frequent complication in the larger goitres for reasons already mentioned. It is necessary to recognize this in the interpretation of the histological conditions in these goitres, as, using mammalian standards, some of the pictures observed could easily be mistaken for sarcoma.

The effect of iodin on the thyroid hyperplasia has been studied in the early, middle and late stages. All stages react with iodin—the mild degrees seemingly more rapidly than the severer degrees. Thus the early stages involute in from 2 to 3 weeks, while the late stages may take 1 to 2 months. This is slower than the iodin reaction time in mammalian hyperplasia. The tumors do not react with iodin as does ordinary hyperplasia. Infection or other complication modifies the reaction. The reaction with iodin is a specific test for functional hyperplasia of the thyroid. As we have not been able to find any stage in the process that does not react with iodin we must conclude that there is no stage of the process that may be looked upon biologically as cancer. Just as iodin invariably stops the hyperplasia and causes the thyroid to involute to the colloid or resting state (from whence it may undergo hyperplasia a second, third or more times, exactly as in mammals) so also spontaneous involution occurs when the fish are transferred to a natural environment. This spontaneous involution has been followed in experiments as well as by means of examples taken at random from the streams. We have never seen an exception except in the case of tumors. As pointed out above recovery does not imply a disappearance of the goitre. It does imply a cessation of growth and the return of the active hyperplasia to the colloid or resting stage.

Thyroid hyperplasia is a compensatory reaction the exact cause of which is still unknown. There is no evidence that it is either infectious or contagious, whether in fish or in man. All the biological data at present available favor the view that fish goitre is common with mammalian goitre is the symptomatic manifestation of a metabolic and nutritional disturbance. There are three major conditions

which in some was (now unknown) influence the thyroid growth, viz., *a limited water supply, overcrowding* and *overfeeding* with a highly artificial and incomplete food.

The water of this hatchery is not intrinsically goitre producing, as the fish will not develop goitre unless at least the factor of overfeeding with an incomplete food operates at the same time. On the other hand they will recover if the overfeeding and overcrowding are corrected, though still living in this same water. It therefore seems probable that the *food* is the major factor acting to bring about a state of nutrition favorable for goitre development. It is impossible at this time to suggest what elements in the food may be at fault, whether it is deficiency or an excess or a disproportion in the relative food values which should be considered as the causal factor or factors. It is of interest to note in this connection that we have tried to rear pups and kittens on this highly artificial diet of liver, lung and heart muscle. Such animals always became rhachitic and goitrous and eventually died unless fed in addition with crushed bone.

CONCLUSIONS.

(1) There is no stage of thyroid hyperplasia in brook trout that may be classified biologically as carcinoma.

(2) The incidence of true carcinoma in fish goitre is not greater than that in mammalian goitre.

(3) There is no evidence that goitre is either infectious or contagious.

(4) Goitre is endemic in all hatcheries where the *salmonidae* are artificially reared. Its severity is quantitatively related to the general hygienic conditions, the food, the water supply and the crowding.

(5) The cause of goitre is unknown, but in all probability depends on a disproportion in, or a lack of, certain of the elements necessary for nutrition.

PART III.

PRACTICAL SUGGESTIONS.

The artificial propagation of game fish in the United States, has under unusually favorable national, state and private conditions of legislation and appropriation, surpassed that of any other country.

This rapid growth and extension of the industry has eclipsed the more fundamental studies of the effect on the fish of these progressively increasing artificial conditions of life. However, the extension and development of fish culture had to be carried to the point where drawbacks, diseases, etc., arise, in order that concrete problems might be differentiated for study and solution. This same sequence must necessarily take place in the development of any industry as is amply demonstrated in the history of our plant and domestic animal industries and perhaps more strikingly to us of this age in the so-called industrial diseases of man as for example the carbon worker's cancer, the phosphorus poisoning of match workers, lead poisoning, etc.

The culture of fish in captivity is one of the newest phases of animal industry and it has only been in the last decade that its clear cut industrial problems have arisen so that today we are at the beginning of the study of the physiological requirements of artificially reared fish as regards food, water, crowding and general sanitary conditions. It is from these highly artificial conditions of *food, water* and *environment* that the disease "Goitre" takes its origin and in this respect it may be classed as an industrial disease. We will discuss briefly on the following pages the etiological factors in this disease and the methods for its cure and prevention in so far as they are at present known.

As may be inferred from parts I and II of this paper, our studies have been confined largely to the brook trout (Char) but as the true trout and salmon belong to the same family (Salmonidae) and are grown under similar conditions of food, water and environment what has been said and that which follows apply to all three species.

(1) *Food.* These fish being carnivora, the most economical and practical food has been liver, heart and lung tissues of either cattle, pigs or sheep. A recent governmental ruling prevents the interstate traffic in lungs and therefore in many hatcheries lung tissue cannot be used. Such a diet is highly artificial. It is in addition a protein diet and therefore low in carbohydrates and certain important salts. It has been shown by Baumann (Zeitschr. f. Physiol. Chem. 1896 XXII, 11) that the thyroids of dogs fed exclusively on a meat diet undergo hyperplasia in a few weeks. This observation was indirectly made earlier at The London Zoological Gardens when, in attempting to rear lion cubs on a raw meat diet, they became rhachitic and goitrous. Chalmers Watson (Proc. Physiol. Soc. Lond. Dec. 17, 1904) has shown that a meat diet produces increased thyroid activity with enlargement in rats. We have also found that young puppies fed with an exclusive meat diet or fed with liver, lung or heart whether boiled or fresh develop rickets with very marked enlargment of their thyroids. Puppies fed with a mixed diet of milk, bread, bone and meat developed normally. Such experiments illustrate the effect of an ex-

clusive meat diet on the thyroid's activity and growth in mammals. With fish the results are even more severe on account of the additional factors of natural voraciousness and excessive feeding as is amply shown by the presence of thyroid hyperplasia (goitre) in all hatcheries where diets of liver, lung and heart are used.

Food is unquestionably the greatest single factor in the etiology of fish goitre. The influence of the food mentioned above in this capacity is divisible into at least four factors, viz:

(a) by reason of its being a highly unnatural food,

(b) by reason of its being fed in excessive amounts,

(c) by reason of its containing an excess of certain elements and a deficiency of other elements necessary for normal nutrition,

(d) by reason of any bacterial decomposition.

(a) The problem of modifying this highly *unnatural food* so as to approach the natural food of these fish is largely an economic one. As it is the most difficult of all the problems to be met, and as it is possible that by solving the more immediate and urgent problems this may cease to be a problem, we will not discuss it here.

(b) The problem of *excessive feeding* is easily met. Fish living in small over crowded ponds with small supplies of fresh water do not require and cannot make use of as large amount of food as fish living wild in the stream. The experiments described in part II of this paper show that one light feeding daily to fish of 1 or more years old is sufficient to insure growth. A restricted diet has many indirect advantages. First, by decreasing the urinary and fecal excretions and by decreasing the amount of uneaten food, a smaller water supply becomes less charged with products of decomposition. Secondly, the putrifactive and fermentative processes in the ponds is lessened and this in turn saves more of the oxygen content of the water for the fish. Thirdly, it would tend to lessen the bacterial flora of the ponds. It need scarcely be added that in certain seasons of the year and in places where the water supply is abundant many of these intoward influences may be thus overcome.

(c) The problems arising out of the fact that a diet of liver, lung and heart is incomplete in that it contains an excess of certain food stuffs and a deficiency of others are more difficult because we do not exactly know what elements are in excess or what elements are deficient. One means of lessening any disproportion between the several elements of nutrition is to restrict the quantity of food, as it is conceivable that the fish in attempting to metabolise those elements present in excess create an extra drain on other elements already deficient or normally derived from another source than the meat.

(d) The feeding of stale and decomposing food ought to be avoided since these fish normally eat the freshest of foods. The bac-

terial decomposition of liver, lung or heart produces protein decomposition products at least foreign and perhaps poisonous to the fish.

(2) *Water supply* derives its signifiance from two other factors viz., the food and the number of fish, for the reason that the major functions of water are (1) to carry oxygen (2) to dissolve and remove effete products and (3) to furnish additional elements of food both organized, as algae, amoeba, crustacea, arthropods etc. and unorganized, as salts in solution. An abundant supply of uncontaminated water is therefore highly important.

(3) *Overcrowding* in turn derives its significance from the water supply and the food. Its major injurious influence may be inferred from what has already been said concerning food and water supply.

(4) *Sanitation* These fish naturally live in unusually clean and unpolluted streams. In the rapid development of fish culture the full significance of these natural surroundings has often been overlooked although in the co-ordinated branches of plant and animal industries we have long recognized the necessity of drainage and cultivation in the case of plants, and of sewage disposal, clean quarters and ample space in the case of mammals. In the case of the fish ponds most of the older ones are small, usually rectangular with planked sides. They are generally used continuously from year to year without periods of drainage, drying, or exposing their bottoms to the action of air and light. Among the older fish of a given hatchery regular removal of the accumulated food, feces and other debris from the ponds is not often practiced. Too often this work is left to be performed by the water—an agency which often labors under the handicaps of being quantitatively inadequate and of mechanical defects of the pond construction. In our limited observations among governmental, state and private hatcheries, it has seemed to us that much improvement could be made with little additional expense.

Taking up the type of pond best suited to overcome certain of the above mentioned objections, it has seemed to us that ponds of the type diagrammatically shown in Figs. 11, 12, 13 would have certain advantages. The roughly boat shaped structure could be made of concrete, brick or lumber. The size could be of any dimensions desired but preferably as long as the space would permit. Such a pond would eliminate the dead corners where fecal matter, etc., accumulate and by constructing a flat bottomed square depression with perpendicular sides just anterior to the outlet all the detritus would be swept back by the water currents and deposited in this "catch basin." From thence it could easily be removed by hand or in places

where the fall would permit a wash-out-pipe could be connected with this "catch basin" which would allow of more complete and easier removal of the detritus. Such a pipe could also be used to drain the pond so that drying and cleaning could be done whenever necessary. Having the bottom of concrete or brick instead of soil, with curved sides sloping toward the centre line and also with curved bottom sloping from the head and the foot of pond to the deepest part in the middle, there would be less danger of the bottoms becoming foul and less favorable conditions for the development of bacteria, fungi and the various forms of animal life that are commonly found in the soil bottom ponds. Such a pond as we have outlined would overcome the major unsanitary conditions common to the old rectangular ponds and render more efficient a smaller water supply especially as regards oxygen. Perhaps many practical changes could be suggested to render this type of pond more efficient and we have only suggested this model as a possible solution for sanitary defects that will have to be met if the artificial culture and storage of these fish is to be extended along the lines now in vogue.

A more practical method of storing these fish would be to make ponds in a stream by means of dams and thus utilize all the water of a larger stream. This idea is already being put to practical use at the Blooming Grove Hunting and Fishing Club's hatchery.

Fig. 2. Normal thyroid from a 29 mos. old brook trout showing the distribution of the follicles, (a) thyroid follicles, (b) aorta, (c) striped muscle bundle, (d) cartilage.

Fig. 3. Developing goitre (active thyroid hyperplasia). Note the enlargement and distortion of the follicles, the regular, uniform columnar epithelium and the great reduction in the colloid content of the follicles.

Fig. 4. The beginning of the process of recovery, (involution) or the return of the active hyperplasia (as shown in Fig.) to the colloid or resting stage. (The elongation of the follicles in this case is due to tension). Note the appearance of stainable colloid in the follicles the contraction of the stroma and early regressive changes in the follicular epithelium.

Fig. 5. Thyroid area of a 5 mos. old brook trout. Note that the entire thyroid area is filled with actively growing thyroid tissue with the usual follicular infoldings and columnar epithelium characteristic of active thyroid hyperplasia, (a) bone and cartilage, (b) pharyngeal mucosa, (c) the two terminal branches of the aorta.

Fig. 6. Colloid goitre—result of iodin treatment of active thyroid hyperplasia. Note the flat cubical epithelium, the rounded colloid containing follicles and the much increased stroma; (a) pharyngeal mucosa, (b) thyroid follicles

Fig. 7. Colloid goitre—spontaneous involution due to residence in the trout stream. Note the similarity of the individual follicles as regards epithelium and colloid to those of Fig. . Note also the extension of the follicles between the muscle fibres (so-called invasion); (a) and (b) thyroid follicles in the striped muscle bundle.

Fig. 8. Colloid goitre—effect of iodin on active hyperplasia. Note the relation of follicles to *bone*. (a) bone, (b) thyroid follicles with normal colloid and epithelium.

Fig. 9. Portion of thyroid area of a 6 mos. old fry showing complete filling of thyroid area with actively hyperplastic thyroid tissue, (a) relation to aorta, (b) relation to bone and cartilage, (c) relation to striped muscle, (d) relation to vein, (e) gill arch.

Fig. 10. Portion of large ventral goitre showing (a) growing border of a thyroid tumor; (b) adjacent thyroid hyperplasia in which the tumor developed.

Fig. 11. Drawing illustrating the general appearance of a pond as seen from above looking directly down.

A—The two screens in "tail board."

B.—"Wash-Out-tube" at the base of "catch basin."

C.—Catch basin.

D.—Out-let for water from pond.

Note the absence of any corners where debris may lodge outside the "catch basin."

Fig. 12. Drawing illustrating longitudinal section of pond.

A.—"Tail board."

B.—"Wash-out-tube."

C.—"Catch basin."

Note the relatively flat bottom and the curved ends.

Fig. 13. Detailed drawing of the tail end of a pond as seen from above and in front.

A.—Screens in tail board.

B.—Wash-out-tube" at base of catch basin.

C.—Catch basin.

D.—Outlet for water from pond.

Note the rounded bottom and sloping sides.

Note also the "tail board" or "Partition" to produce a status of the water which debris may settle into the catch basin at its base.

Note: This study was made possible through facilities granted by and through the co-operation of the Honorable W. E. Meehan, Commissioner of Fisheries for the State of Pennsylvania. We wish to express our obligations and thanks to Mr. Meehan, to the Officers of Blooming Grove Fishing & Hunting Club, and to Mr. Florance Rolonson, Superintendent at the hatchery, for their aid and interest.

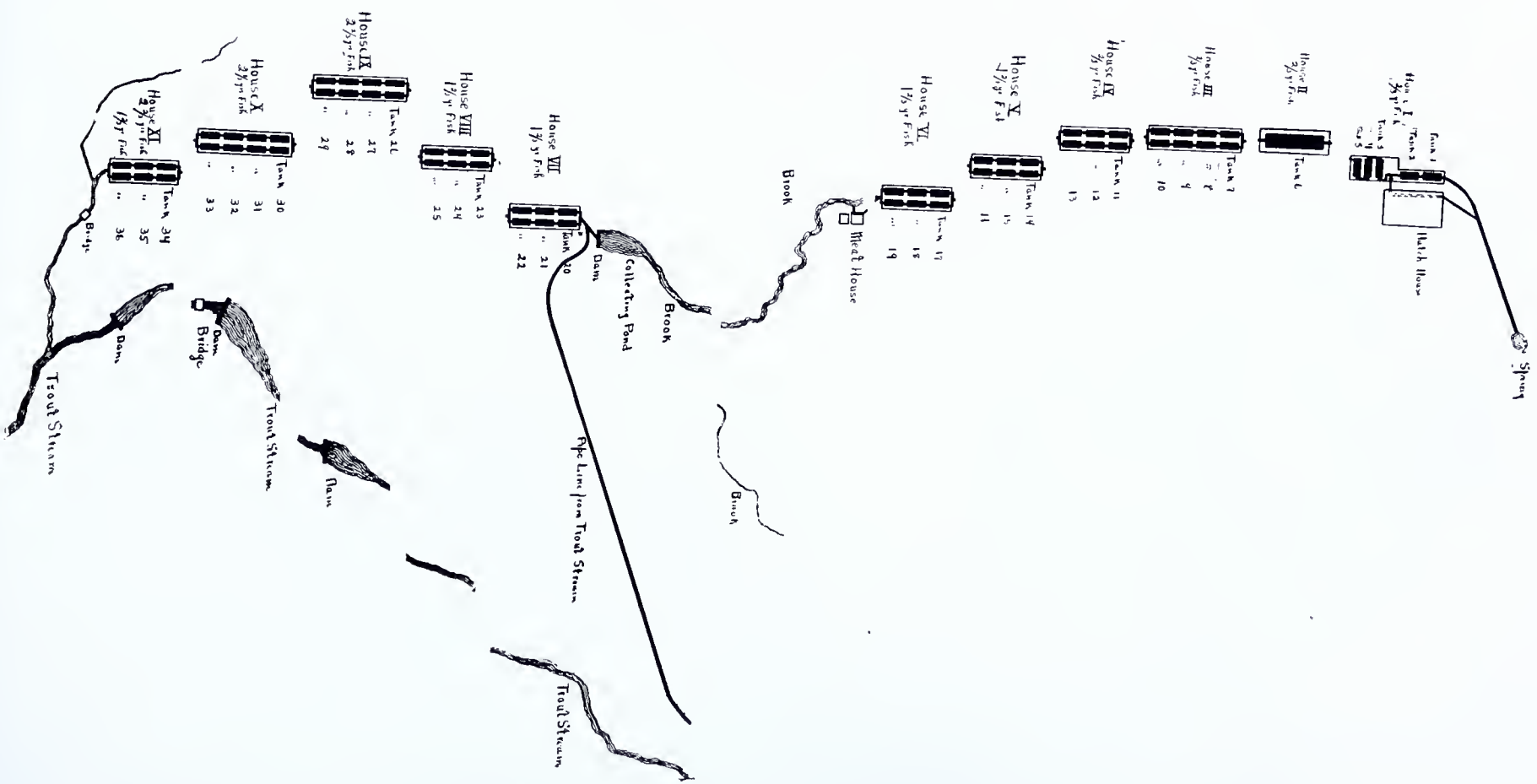
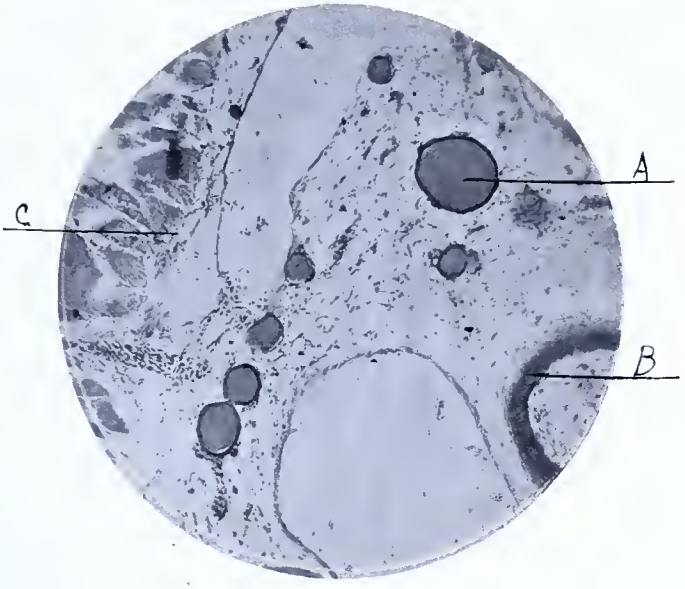


Fig. 1. Semidiagrammatic drawing of the plan, arrangements and location of the hatchery, water supplies, houses and retaining tanks. This plate is taken from the previous bulletin No. 7. The lines indicating the age of the fish in the various houses are no longer necessary for the press matter. The note referring to location marked * is on the second stream from the right of the cut marked trout stream.



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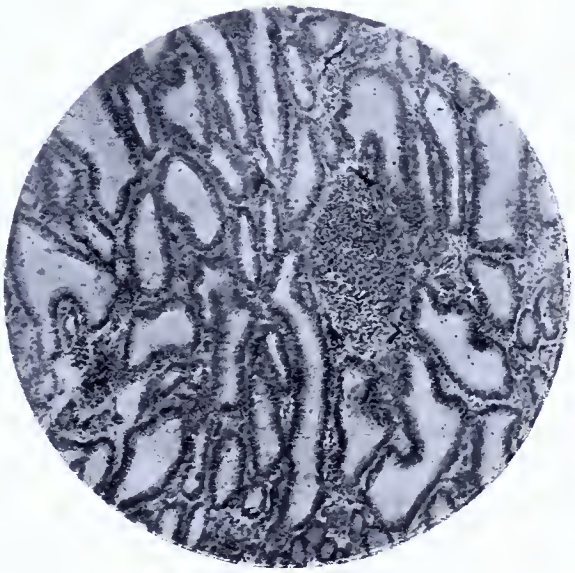

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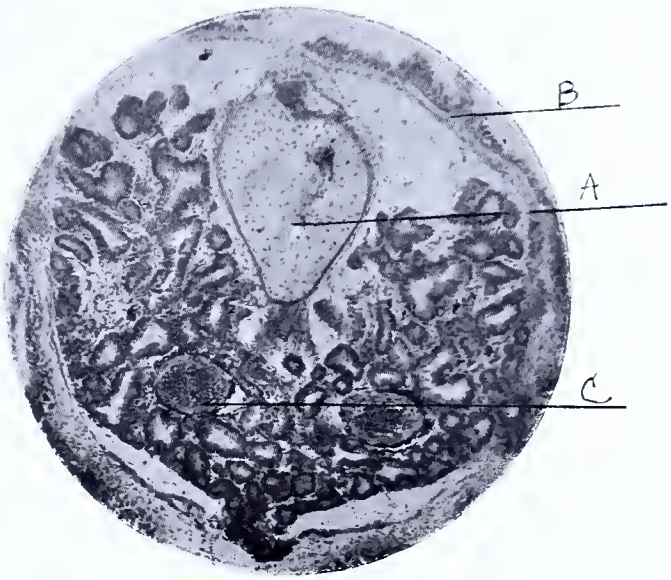

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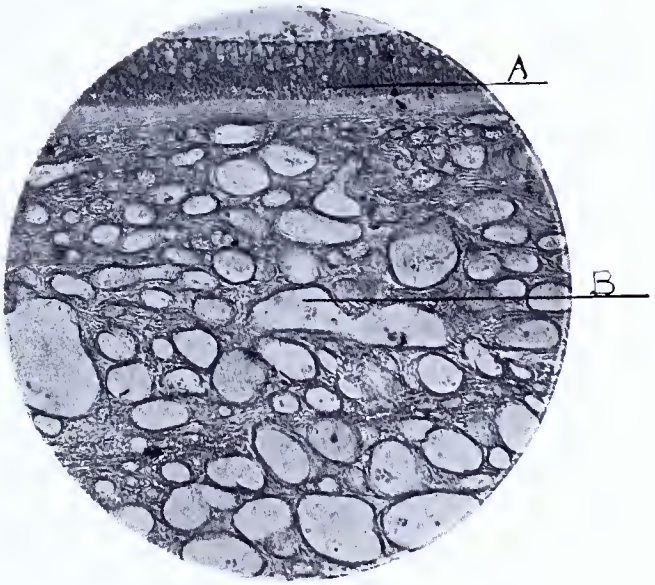

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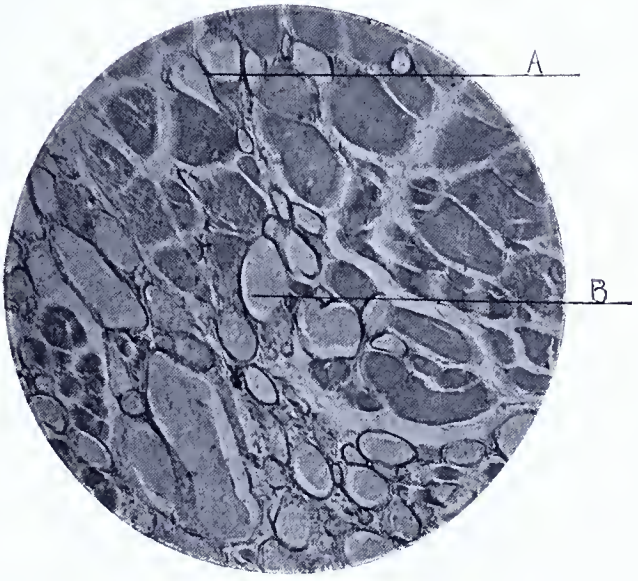

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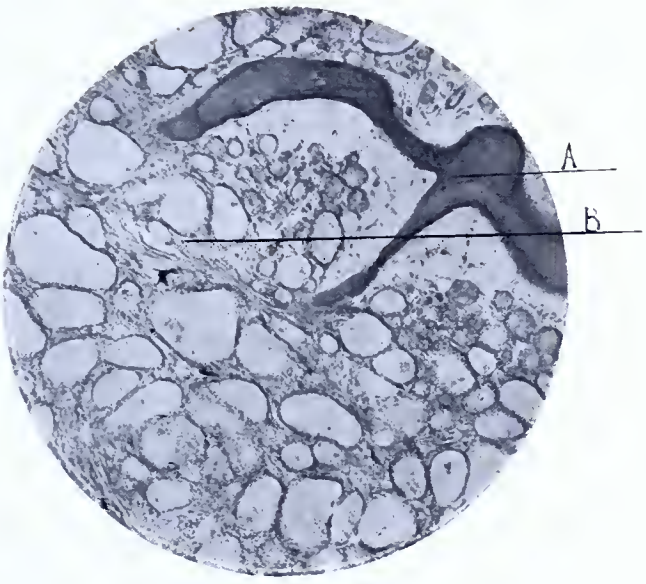
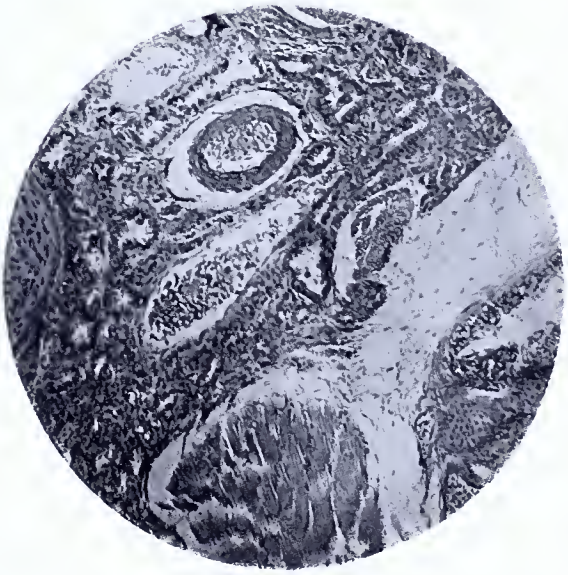


Fig. 8



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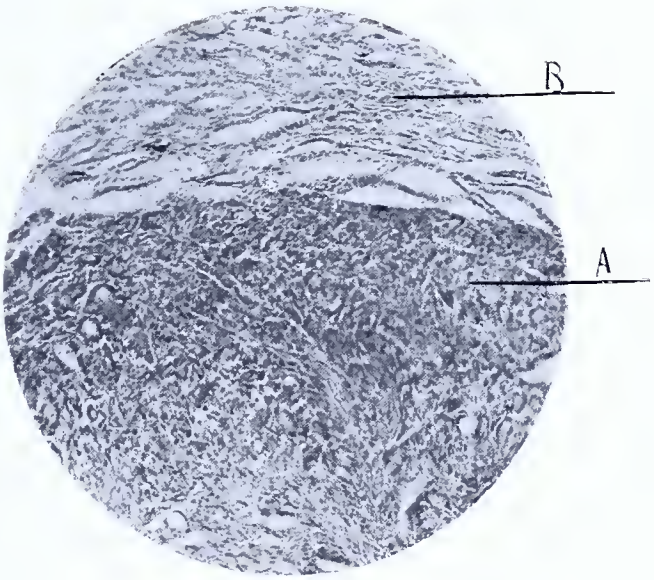

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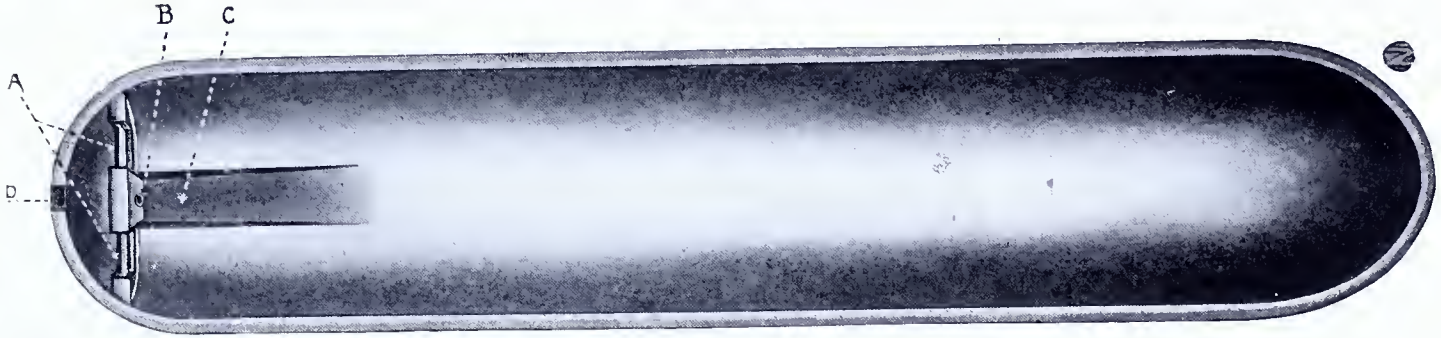
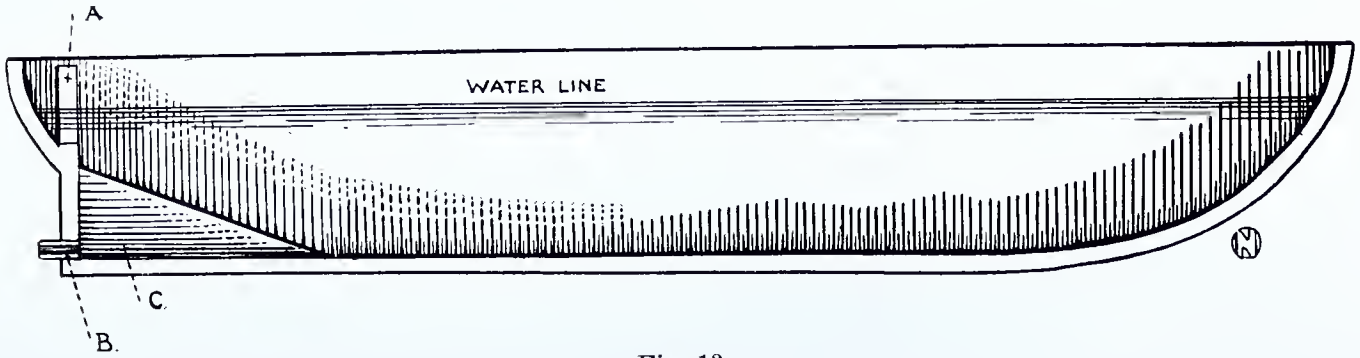


Fig. 11.



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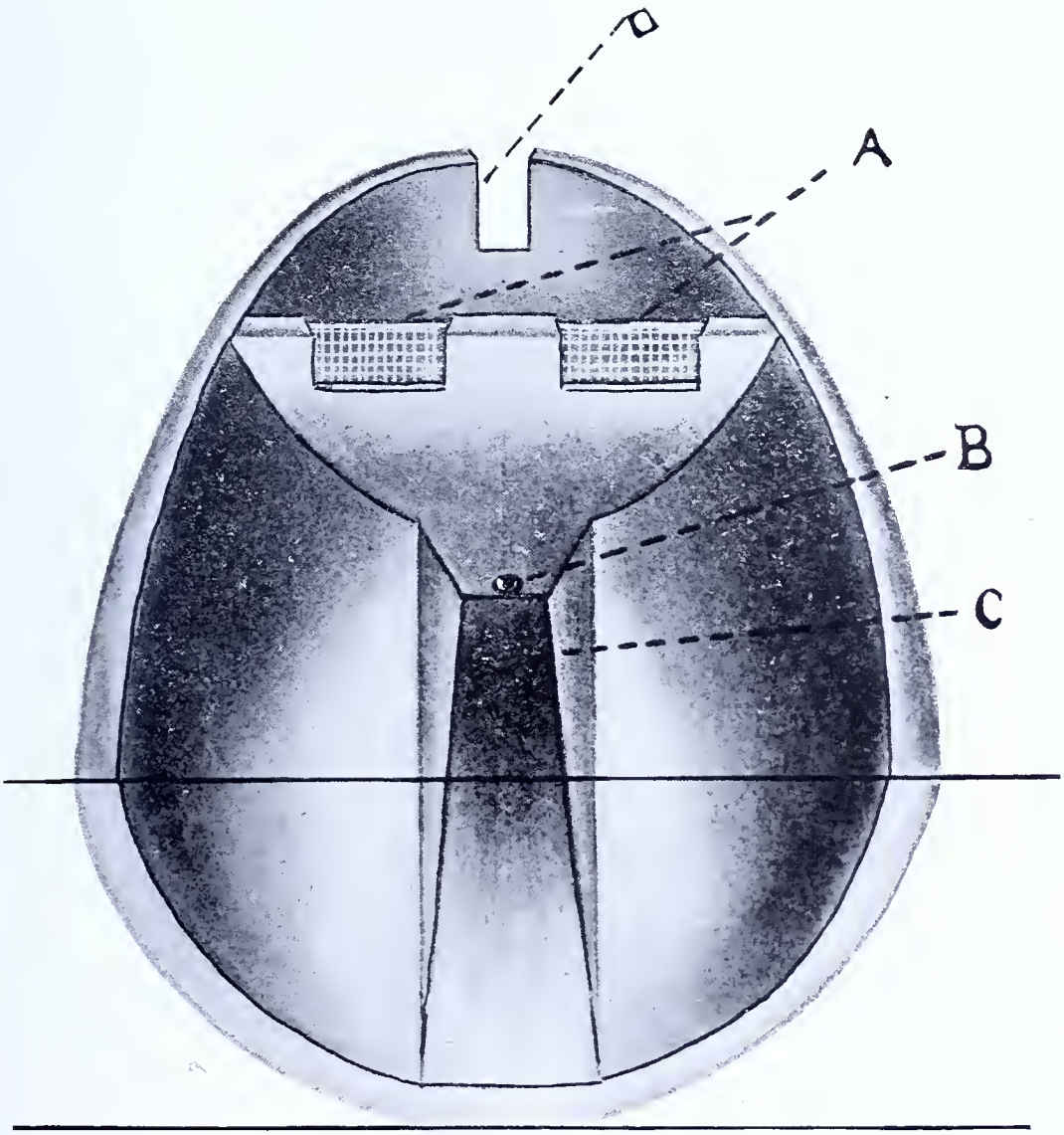

Fig. 13.